

TITLE: COMPOUNDS AND METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE

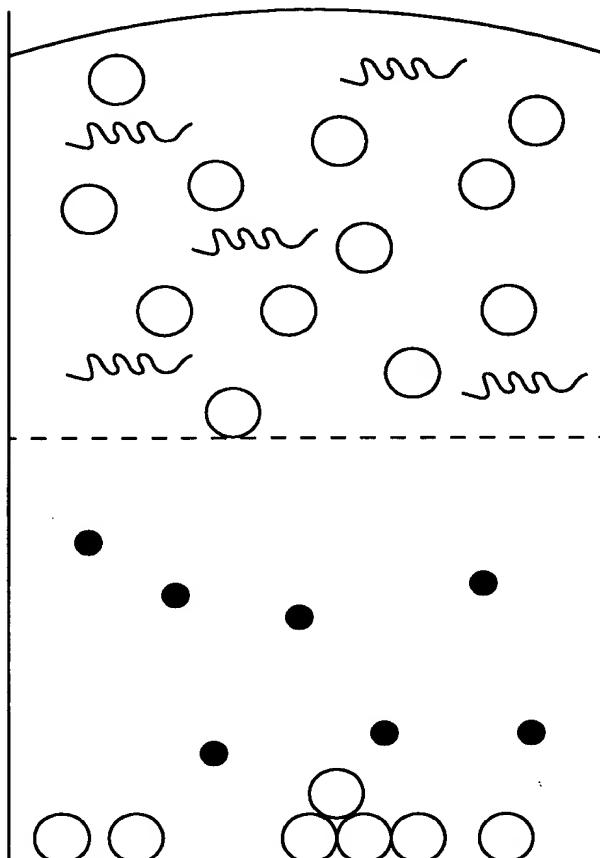
INVENTOR'S NAME: David J. Grainger, et al.

SERIAL NO.: 09/150,813 DOCKET NO.: 1543.002US1

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TOP COMPARTMENT



LOWER COMPARTMENT

FIG. 1

TITLE: COMPOUNDS AND METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE

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EFFECTS OF PEPTIDE 3 WITH MCP-1 (50ng/ml)

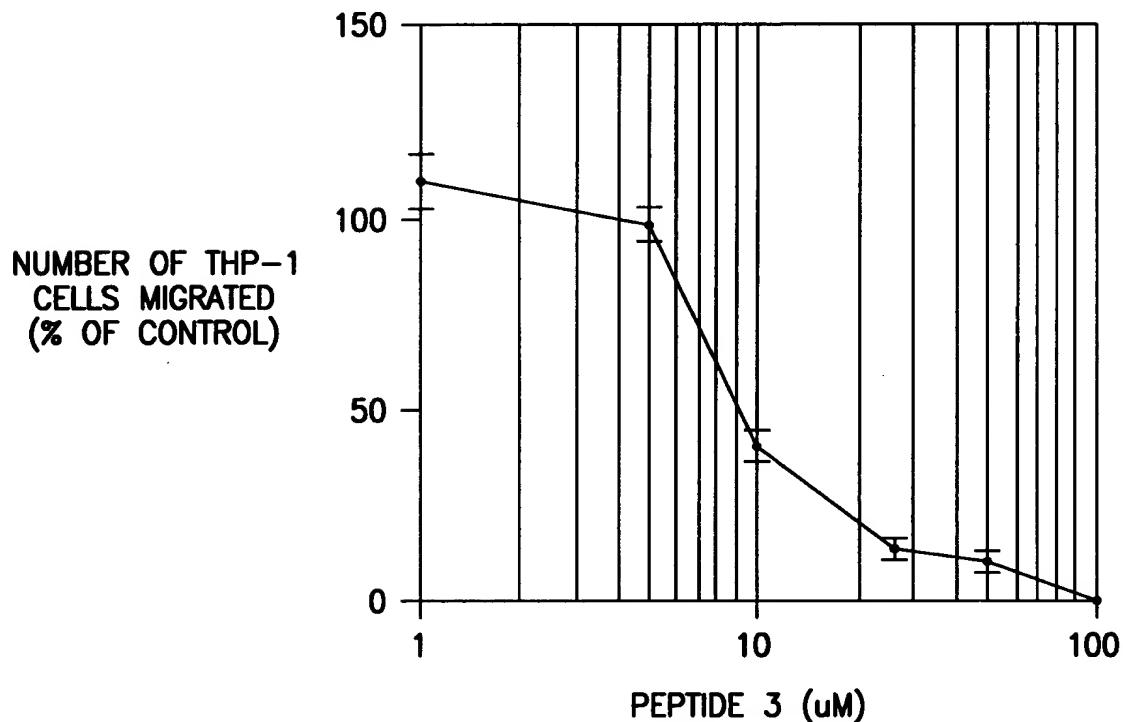


FIG. 2

TITLE: COMPOUNDS AND METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE

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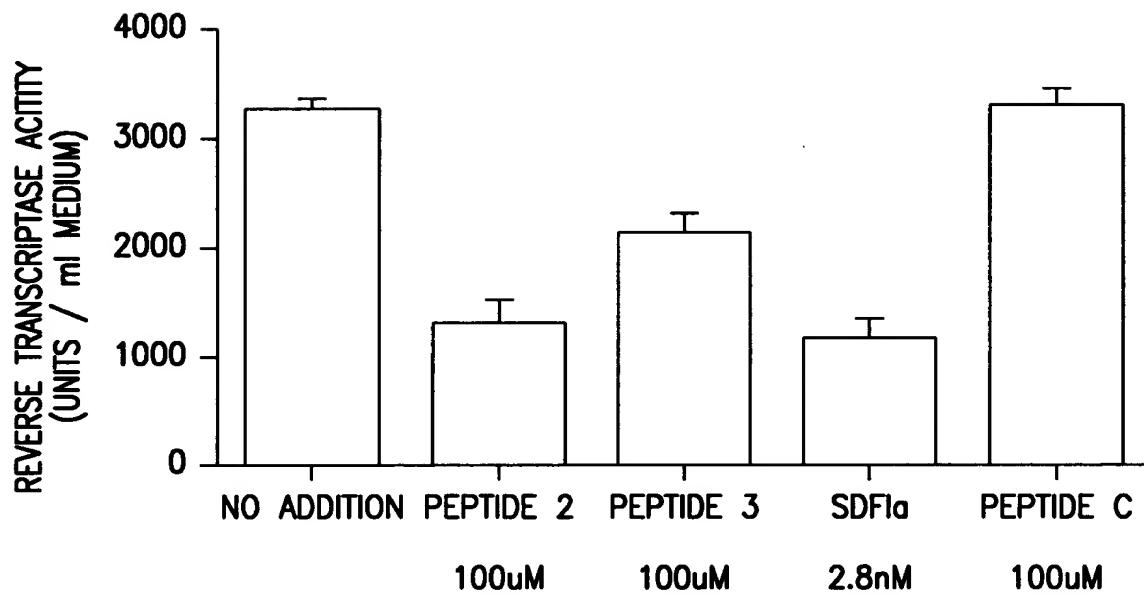


FIG. 3

TITLE: COMPOUNDS AND METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE

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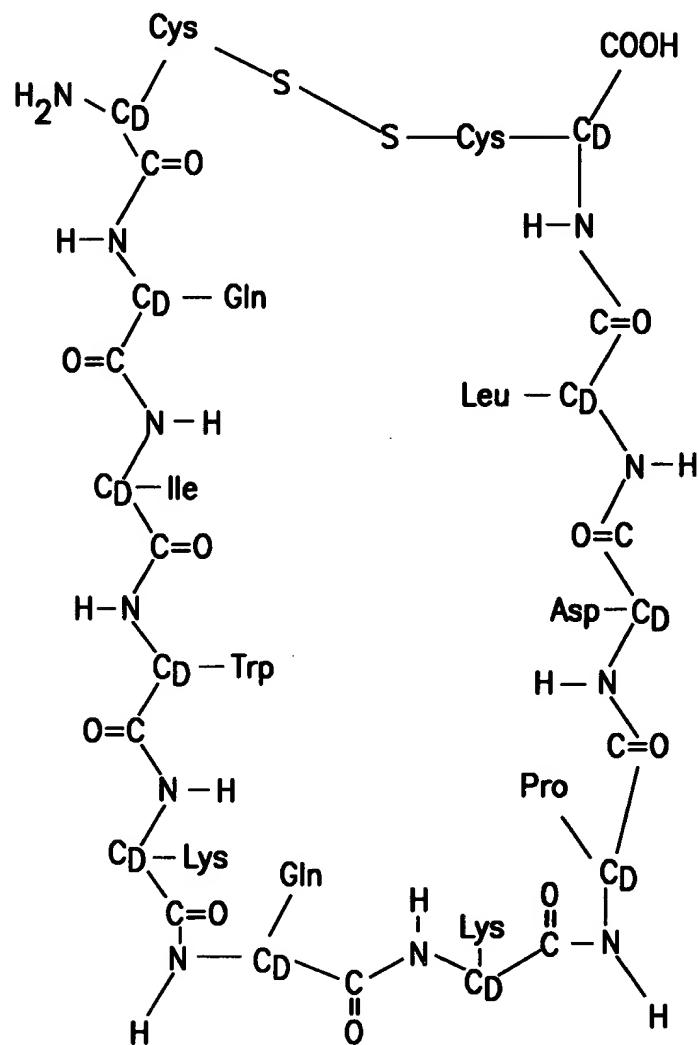


FIG. 4

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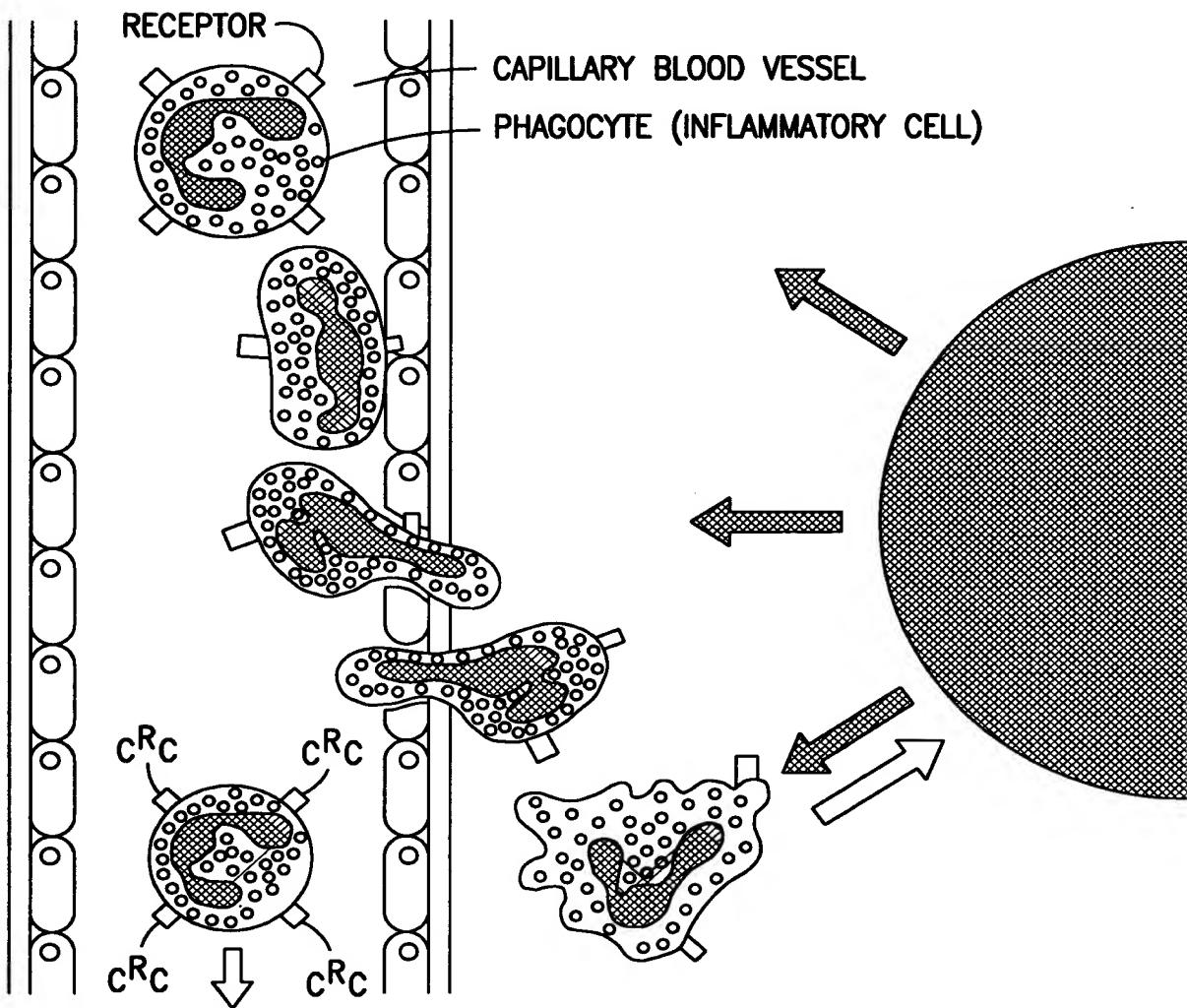


FIG. 5

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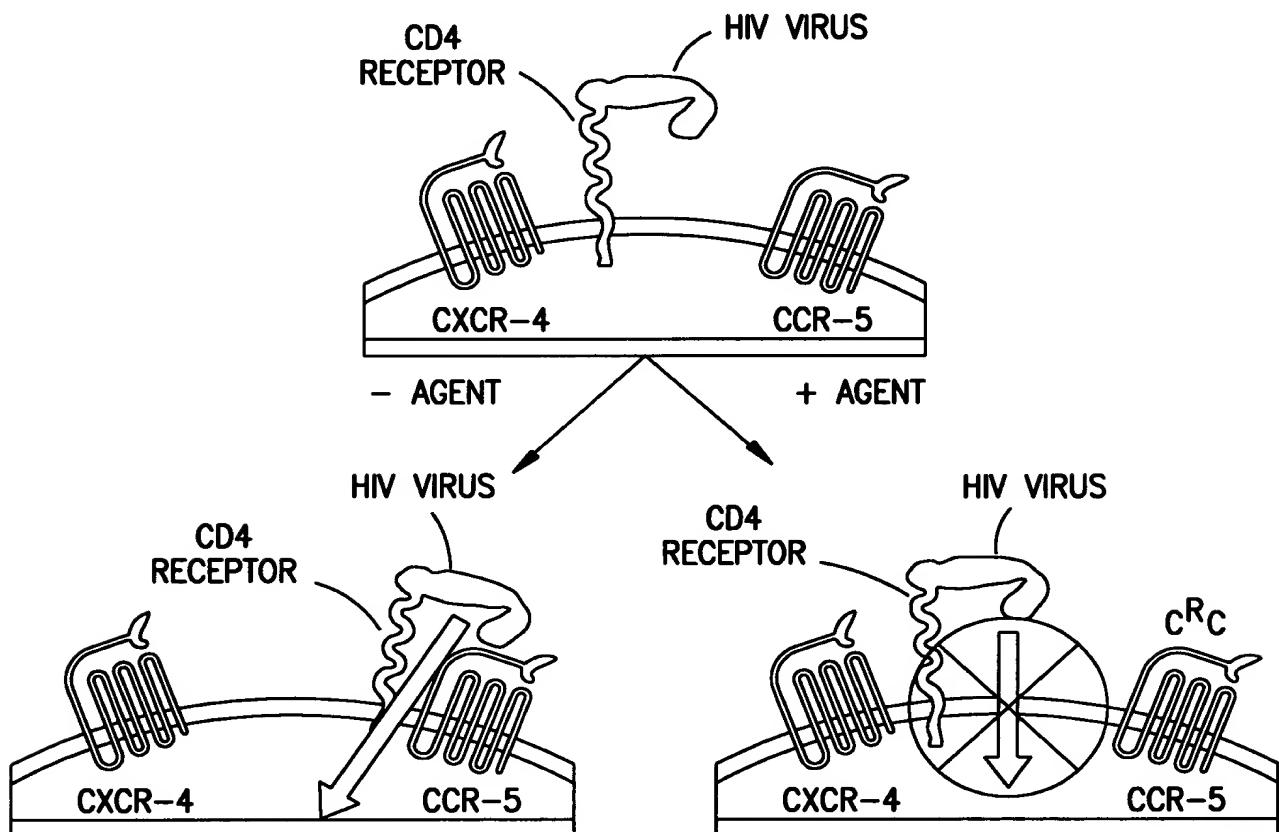


FIG. 6



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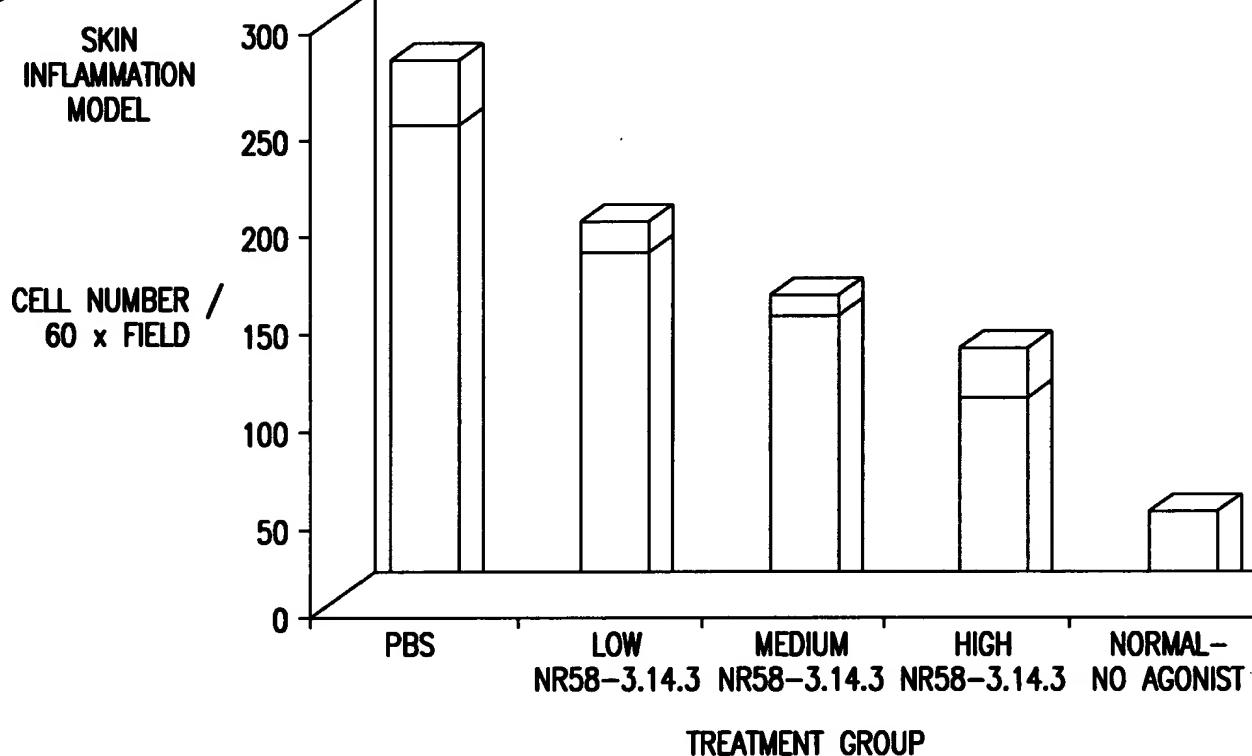


FIG. 7A

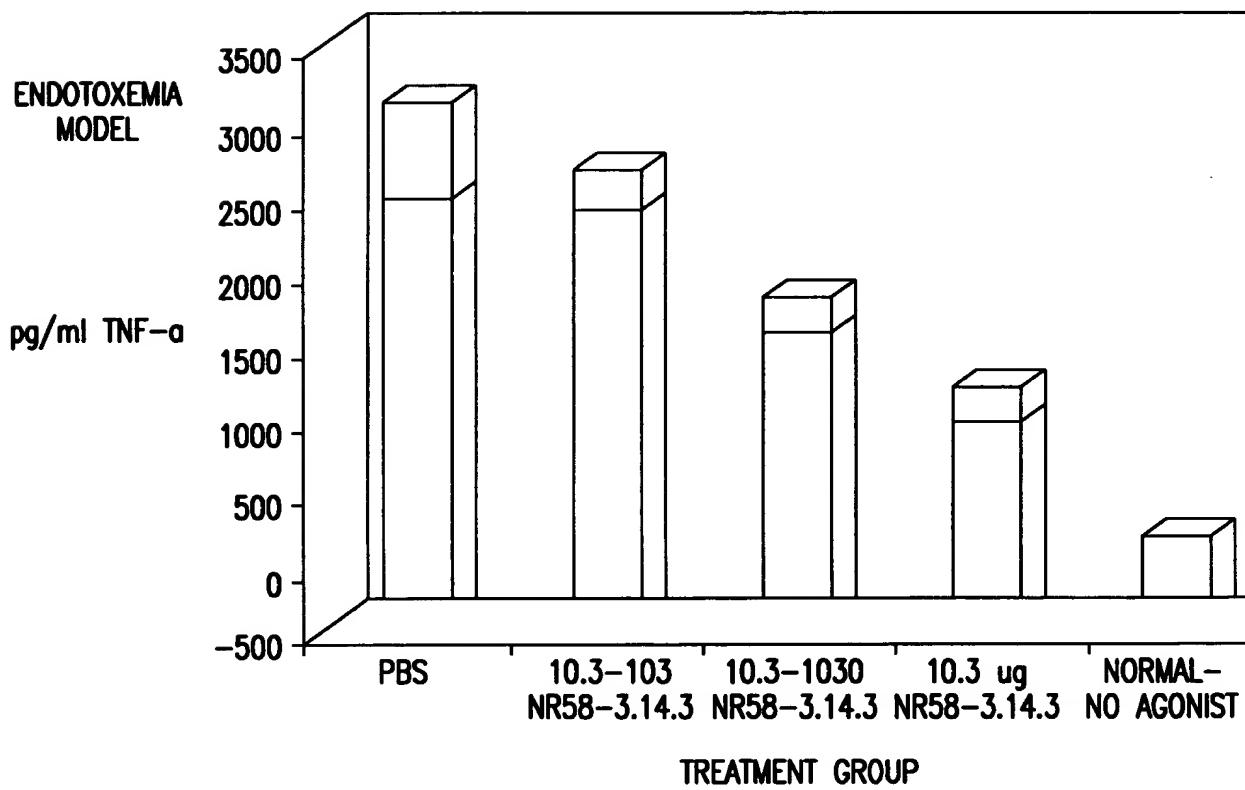


FIG. 7B

TITLE: COMPOUNDS AND METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE

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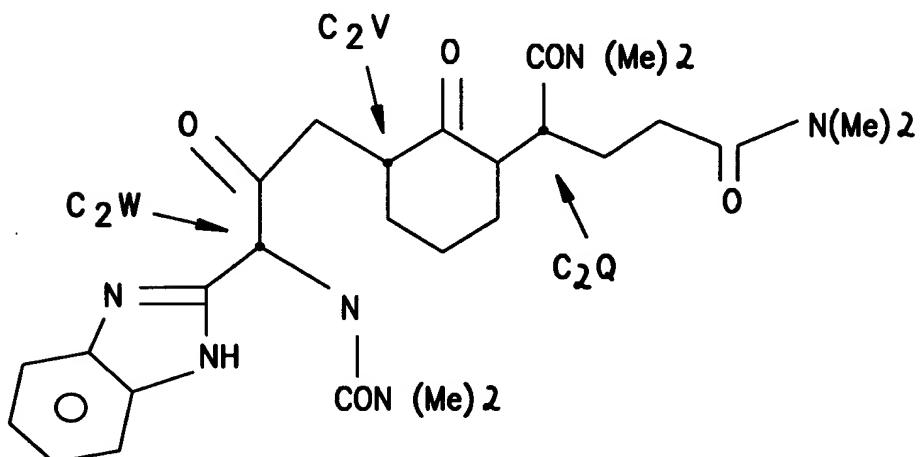


FIG. 8

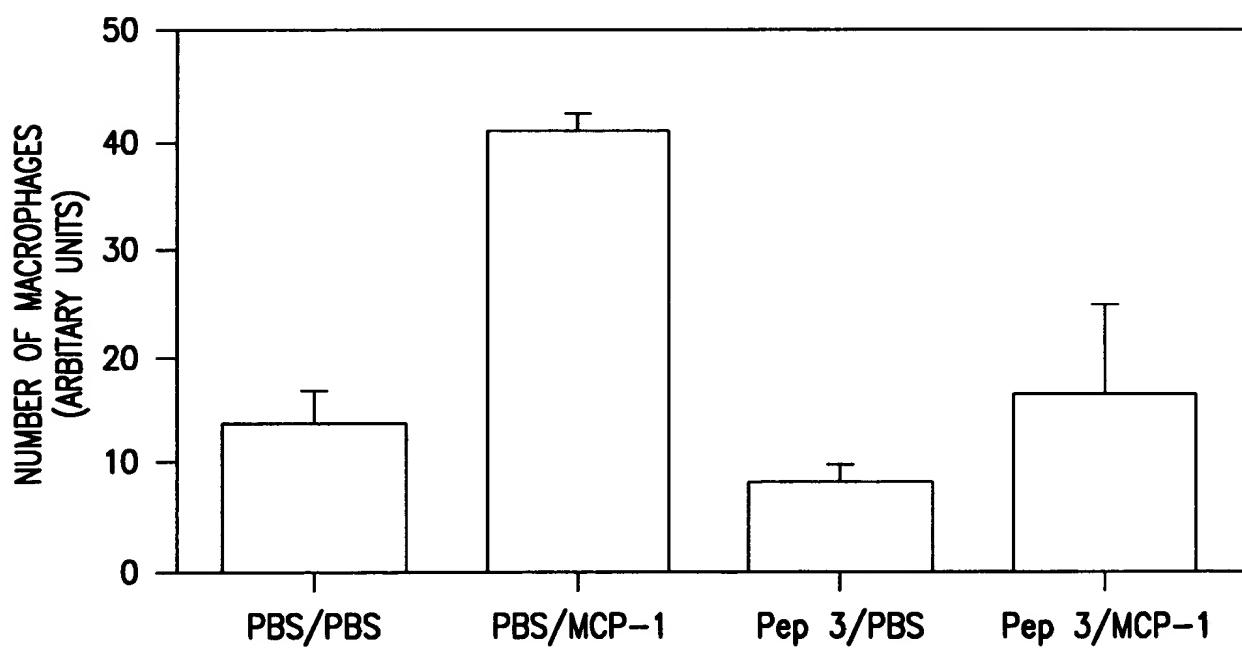
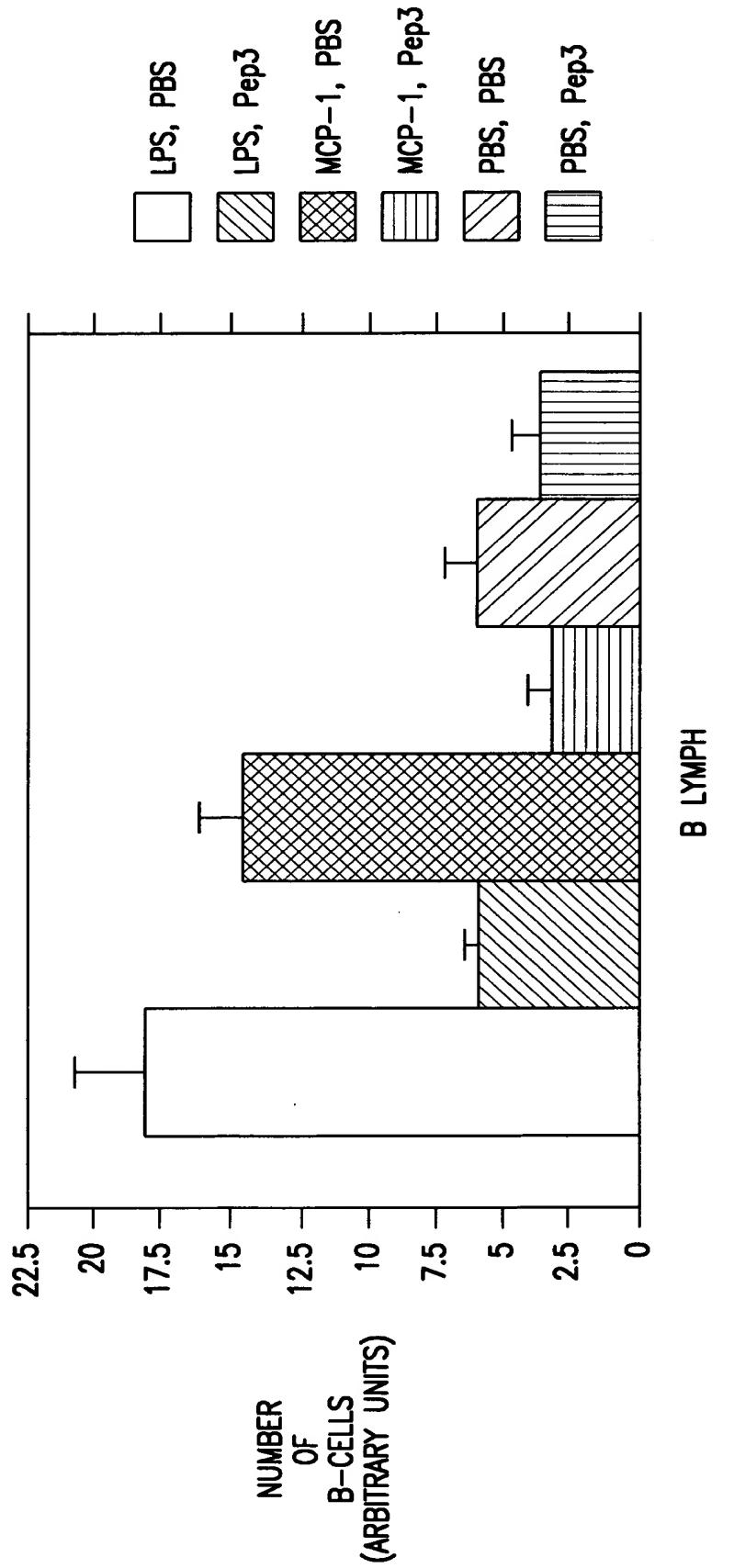


FIG. 9

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B LYMPH
FIG. 10

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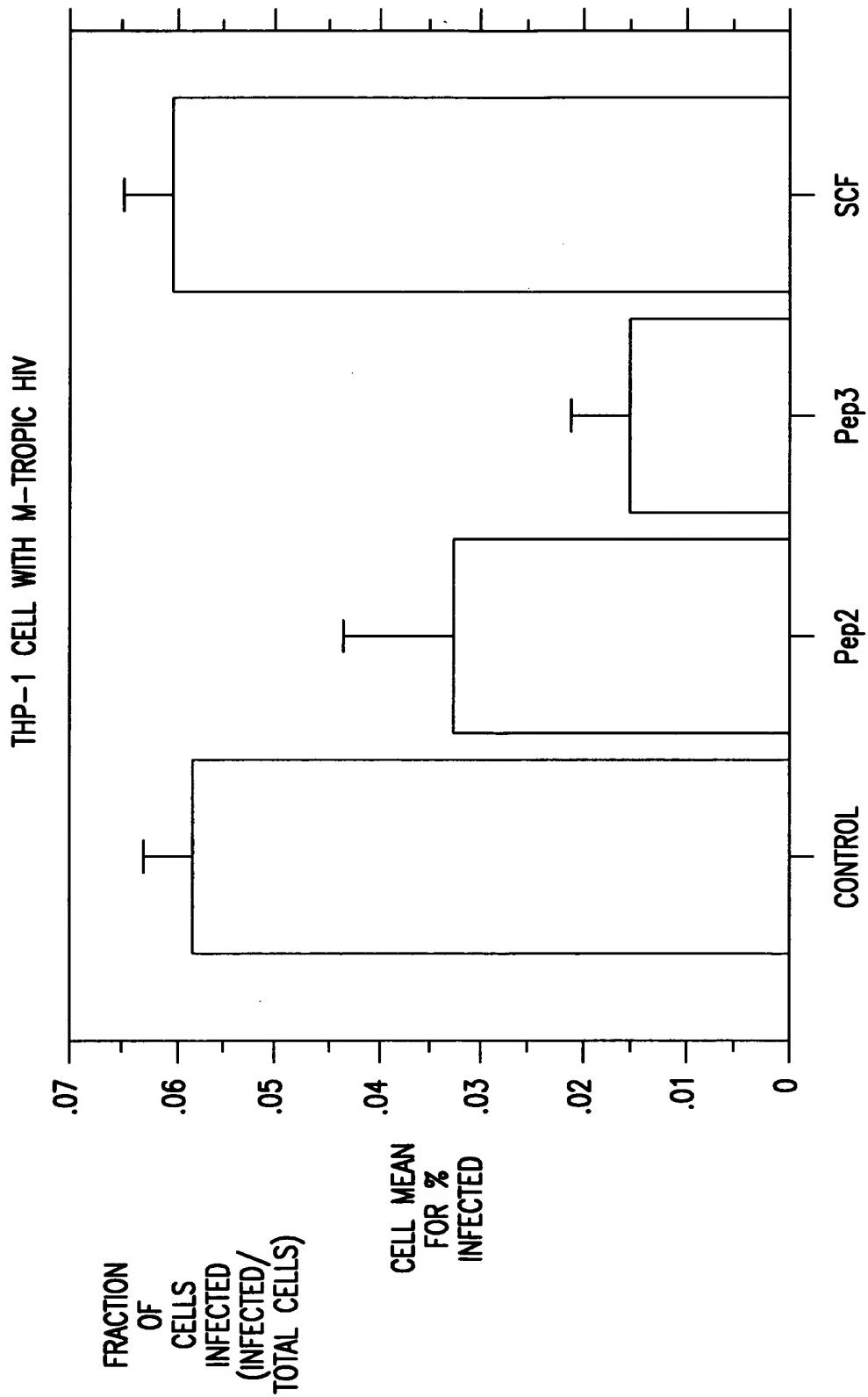


FIG. 11



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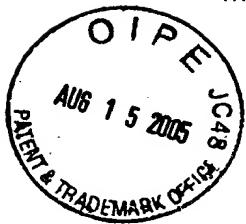
<u>Amino Acid</u>	<u>Codon</u>
Phe	UUU, UUC
Ser	UCU, UCC, UCA, UCG, AGU, AGC
Tyr	UAU, UAC
Cys	UGU, UGC
Leu	UUA, UUG, CUU, CUC, CUA, CUG
Trp	UGG
Pro	CCU, CCC, CCA, CCG
His	CAU, CAC
Arg	CGU, CGC, CGA, CGG, AGA, AGG
Gln	CAA, CAG
Ile	AUU, AUC, AUA
Thr	ACU, ACC, ACA, ACG
Asn	AAU, AAC
Lys	AAA, AAG
Met	AUG
Val	GUU, GUC, GUA, GUG
Ala	GCU, GCC, GCA, GCG
Asp	GAU, GAC
Gly	GGU, GGC, GGA, GGG
Glu	GAA, GAG

FIG. 12

TITLE: COMPOUNDS AND METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE

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Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro	pro
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala	leu
Pro (P)	gly	gly
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

FIG. 13



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REPLACEMENT SHEET

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PEPTIDE-3

LFL peptide 3(1-12)[MCP-1]: Residues 50-61 of mature hMCP-1
E-I-C-A-D-P-K-Q-K-W-V-Q (SEQ. ID. NO.: 1)
L amino acids

LFL peptide 3(3-12)[MCPI] Residues 52-61 of mature hMCP-1
C-A-D-P-K-Q-K-W-V-Q (SEQ. ID. NO.: 7)
L amino acids

LFL peptide 3(1-6)[MCP1]: Residues 50-55 of mature hMCP-1
E-I-C-A-D-P (SEQ. ID. NO.: 8)
L amino acids

LFL peptide 3(7-12)[MCP1]: Residues 56-61 of mature hMCP-1
K-Q-K-W-V-Q (SEQ. ID. NO.: 9)
L amino acids

LFL Leu₄peptide3(1-12)[MCP-1]
E-I-C-L-D-P-K-Q-K-W-V-Q (SEQ. ID. NO.: 10)
L amino acids

LFL Ser₇peptide3(1-12)[MCP-1]
E-I-C-A-D-P-S-Q-K-W-V-Q (SEQ. ID. NO.: 11)
L amino acids

LFL Ile₁₁peptide3(1-12)[MCP-1]
E-I-C-A-D-P-K-Q-K-W-I-Q (SEQ. ID. NO.: 13)
L amino acids

LFL Leu₄Ile₁₁peptide3(1-12)[MCP-1]
E-I-C-L-D-P-K-Q-K-W-I-Q (SEQ. ID. NO.: 14)
L amino acids

CFL Cys₉Leu₄Ile₁₁Cys₁₃peptide3(1-12)[MCP-1]
C-E-I-C-L-D-P-K-Q-K-W-I-Q-C (SEQ. ID. NO.: 106)
L amino acids

LRD Leu₄Ile₁₁ peptide 3(1-12)[MCP-1]
q-i-w-k-q-k-p-d-l-c-i-e
D amino acids

FIG. 14A

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CRD Cys₀Leu₄Ile₁₁Cys₁₃peptide 3(1-12)[MCP-1]
c-q-i-w-k-q-k-p-d-l-c-i-e-c
D amino acids

LFL Ser₇Glu₈Glu₉peptide3(1-12)[MCP1]:Residues 50-61 of mature hMIP1 α
E-I-C-A-D-P-S-E-E-W-V-Q (SEQ. ID. NO.: 12)
L amino acids

LFL peptide3(10-12)[MCP-1]
W-V-Q
L amino acids

CFL Cys₀Cys₄ peptide3(10-12)[MCP-1]
C-W-V-Q-C (SEQ. ID. NO.: 107)
L amino acids

LRD peptide3(10-12)[MCP-1]
q-v-w
D amino acids

LFL peptide3(7-9)[MCP-1]
K-Q-K
L amino acids

LRD peptide3(7-9)[MCP-1]
k-q-k
D amino acids

LFL peptide 3(7-9)[MIP1 α](MIP1 α specific inhibitor)
S-E-E
L amino acids

LRD peptide3(7-9)[MIP1 α] (MIP1 α specific inhibitor)
e-e-s
D amino acids

LFL peptide3(7-9)[IL-8](IL-8 specific inhibitor)
K-E-N
L amino acids

LRD peptide3(7-9)[IL-8](IL-8 specific inhibitor)
n-e-k
D amino acids

FIG. 14B



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LFL peptide₃₍₇₋₉₎[SDF-1 α](SDF-1 α specific inhibitor)

K-L-K

L amino acids

LRD peptide₃₍₇₋₉₎[SDF1 α] (SDF-1 α specific inhibitor)

k-l-k

D amino acids

LFL Leu₄Ile₁₁Cys₁₃ peptide₃₍₃₋₁₂₎[MCP-1]

L-D-P-K-Q-K-W-I-Q-C (SEQ. ID. NO.: 84)

L amino acids

CRD Leu₄Ile₁₁Cys₁₃ peptide₃₍₃₋₁₂₎[MCP-1]

c-q-i-w-k-q-k-p-d-l-c

D amino acids

³H-Ala CRD-Leu₄Ile₁₁Cys₁₃ peptide 3(3-12)[MCP-1](D-Ala attached to Asp residue of CRD-Leu₄Ile₁₁Cys₁₃ peptide 3(3-12)[MCP-1])

³H-L-Leu LRD Cys₁₃ peptide₃₍₃₋₁₂₎[MCP-1]

c-q-i-w-k-q-k-p-d-L-c

D and L amino acids

LFL SES

S-E-S

L amino acids

LFL KKK

K-K-K

L amino acids

LFL Cys₄ peptide₃₍₁₀₋₁₂₎[MCP-1]

W-V-Q-C (SEQ. ID. NO.: 85)

L amino acids

LRD Cys₄ peptide₃₍₁₀₋₁₂₎[MCP-1]

c-q-v-w

D amino acids

LFL Ile₁₁Cys₁₃ peptide₃₍₁₀₋₁₂₎[MCP-1]

W-I-Q-C (SEQ. ID. NO.: 86)

L amino acids

FIG. 14C



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LRD Cys₁₃Ile₁₁peptide3(10-12)[MCP-1]

cqiw

D amino acids

LRD peptide3(7-12)[MCP-1]

q-v-w-k-q-k

D amino acids

CFL Cys₀Cys₁₃peptide3(7-12)[MCP-1]

C-K-Q-K-W-V-Q-C (**SEQ. ID. NO.: 108**)

L amino acids

CRD Cys₀Cys₁₃peptide3(7-12)[MCP-1]

c-q-v-w-k-q-k-c

D amino acids

LFL peptide3(10-12)[RANTES]

WVR

L amino acids

LRD peptide3(10-12)[RANTES]

rvw

D amino acids

LFL peptide3(10-12)[SDF-1]

W-I-Q

L amino acids

Peptide 2

LFL peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1

S-Y-R-R-I-T-S-S-K-C-P-K-E-A-V (**SEQ. ID. NO.: 105**)

L amino acids

CFL Cys₀Cys₁₃peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1

C-S-Y-R-R-I-T-S-S-K-C-P-K-E-A-V-C (**SEQ. ID. NO.: 109**)

L amino acids

LRD peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1

v-a-e-k-p-c-k-s-s-t-i-r-r-y-s

D amino acids

FIG. 14D

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CRD Cys₀Cys₁₆peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1
c-v-a-e-k-p-c-k-s-s-t-i-r-r-y-s-c
D amino acids

LFL peptide 2(1-15)[SDF1]: Residues 26-40 of mature hSDF-1 β
H-L-K-I-L-N-T-P-N-C-A-L-Q-I-V (**SEQ. ID. NO.: 103**)
L amino acids

CFL Cys₀Cys₁₆peptide 2(1-15)[SDF1]: Residues 26-40 of mature hSDF-1 β
C-H-L-K-I-L-N-T-P-N-C-A-L-Q-I-V-C (**SEQ. ID. NO.: 110**)
L amino acids

LRD peptide 2(1-15)[SDF1]: Residues 26-40 of mature hSDF-1 β
v-i-q-l-a-c-n-p-t-n-l-i-k-l-h
D amino acids

CRD Cys₀Cys₁₆peptide 2(1-15)[SDF1]: Residues 26-40 of mature hSDF-1 β
c-v-i-q-l-a-c-n-p-t-n-l-i-k-l-h-c
D amino acids

LFL peptide 2(1-14)[MIP-1 α]: Residues 28-41 of hMIP-1 α
D-Y-F-E-T-S-S-Q-C-S-K-P-G-V (**SEQ. ID. NO.: 5**)
L amino acids

LRD peptide 2(1-14)[MIP1 α]: Residues 28-41 of mature hMIP1 α
v-g-p-k-s-c-q-s-s-t-e-f-y-d
D amino acids

LFL peptide 2(1-16)[IL8]: Residues 27-42 of mature hIL8
E-L-R-V-I-E-S-G-P-H-C-A-N-T-E-I (**SEQ. ID. NO.: 6**)
L amino acids

LFL Peptide 2(1-10)[MCP-1]: Residues 28-37 of hMCP-1
S-Y-R-R-I-T-S-S-K-C (**SEQ. ID. NO.: 87**)
L amino acids

LFL peptide 2(10-15)[MCP-1]: Residues 37-42 of hMCP-1
C-P-K-E-A-V (**SEQ. ID. NO.: 88**)
L amino acids

LFL peptide 2(1-5)[MCP-1]: Residues 28-32 of hMCP-1
S-Y-R-R-I (**SEQ. ID. NO.: 89**)
L amino acids

FIG. 14E

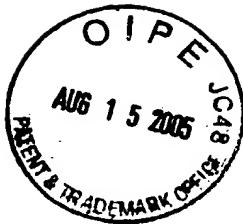
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LFL peptide 2(6-10)[MCP-1]: Residues 33-37 of hMCP-1

T-S-S-K-C (**SEQ. ID. NO.: 90**)

L amino acids

LFL peptide 2(1-9)[MIP-1 α]: Residues 28-36 of hMIP-1 α

D-Y-F-E-T-S-S-Q-C (**SEQ. ID. NO.: 91**)

L amino acids

LFL peptide 2(9-14)[MIP-1 α]: Residues 36-41 of hMIP-1 α

C-S-K-P-G-V (**SEQ. ID. NO.: 92**)

L amino acid

LFL Cys₀Ser₁₀Cys₁₆peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1

C-S-Y-R-R-I-T-S-S-K-S-P-K-E-A-V-C (**SEQ. ID. NO.: 93**)

L amino acids

CFL Cys₀Ser₁₀Cys₁₆peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1

C-S-Y-R-R-I-T-S-S-K-S-P-K-E-A-V-C (**SEQ. ID. NO.: 111**)

L amino acids

LRD Cys₀Ser₁₀Cys₁₆peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1

c-v-a-e-k-p-s-k-s-s-t-i-r-r-y-s-c

D amino acids

CRD Cys₀Ser₁₀Cys₁₆peptide 2(1-15)[MCP-1]: Residues 28-42 of hMCP-1

c-v-a-e-k-p-s-k-s-s-t-i-r-r-y-s-c

D amino acids

FIG. 14F

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THP-1 MIGRATION

SEQUENCE	DARC BINDING	MCP-1	MIP-1 α	SDF-1 α
SYRRITSSKCPKEAV	350nM	ns	ns	ns
VAEKPCPKSSTIRRYS	18 μ M	ns	ns	ns
SYRRITSK	22 μ M	ns	ns	ns
SYRRI	>100 μ M	ns	ns	ns
TSSKC	>100 μ M	ns	ns	ns
CPKEAV	>100 μ M	ns	ns	ns
HLKILNTPNCALQIV	19 μ M	10 μ M	40 μ M	7 μ M
DYFETSSQCSKPGV	>100 μ M	ns	ns	ns
VGPKSCQSSTEFYD	>100 μ M	ns	ns	ns
DYFETSSQC	>100 μ M	ns	ns	ns
CSKPGV	>100 μ M	ns	ns	ns

FIG 15

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SEQUENCE	MOL WT.	DUFFY BINDING BD-50	MCP-1 ED-50	MIP-1 α ED-50	RANTES ED-50	SDF-1 α ED-50	IL-8 ED-50	OTHER DATA
AQPDAINAPVTTC	1302	90 μ M	ns	ns	-	ns	ns	
SYRRITSSKCPKEAV	1725	100 μ M	ns	ns	-	ns	-	
VAEKPKCKSSTIRRYS	1725	18 μ M	ns	ns	-	ns	-	
HLKLINTPNCALQIV	1677.3	19 μ M	10 μ M	40 μ M	-	7 μ M	-	
DYFETSSQCSKPGV	1549	>100 μ M	ns	ns	-	ns	-	
VQPKSCQSSTEFYD	1549	>100 μ M	ns	ns	-	ns	-	
SYRRITSSKC	1097.4	22 μ M	ns	ns	-	ns	-	
CPKEAV	645.8	>100 μ M	ns	ns	-	ns	-	
SYRRI	693.9	>100 μ M	ns	ns	-	ns	-	
TSSKC	525.7	>100 μ M	ns	ns	-	ns	-	
DYFETSSQC	1079.2	>100 μ M	ns	ns	-	ns	-	
CSKPGV	589.8	>100 μ M	ns	ns	-	ns	-	

FIG. 16A

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	1445	6 μ M	8 μ M	7.5 μ M	-	13.5 μ M	10 μ M
CADPKQKNWQ	1202	-	8 μ M	6.5 μ M	-	9 μ M	8.5 μ M
CQWKQKPDAQ	1305	3 μ M	100nM	-	-	-	-
CQWKQKPDAQ	1305	40 μ M	30nM	-	-	-	-
BICADP	647	-	25 μ M	20 μ M	-	18.5 μ M	16 μ M
KQKWWQ	816	15 μ M	7 μ M	5 μ M	-	5.5 μ M	5 μ M
BICDPKQKWWQ	1487	-	8 μ M	7 μ M	-	2.5 μ M	3 μ M
EICADPSQKWWQ	1404	25 μ M	7 μ M	5.5 μ M	-	4 μ M	3 μ M
EICADPKQKWWQ	1459	-	5.5 μ M	3.5 μ M	-	7 μ M	2 μ M
EICDPKQKWWQ	1501	90 μ M	2 μ M	2 μ M	-	4 μ M	3.5 μ M
WQ	431.5	1 μ M	8 μ M	7.5 μ M	1.5 μ M	2.25 μ M	1 μ M
KQK	464.5	50 μ M	7 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
SEE	399.4	>100 μ M	>100 μ M	-	>100 μ M	>100 μ M	>100 μ M
KEN	425.4	>100 μ M	-				

FIG. 16B

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	KLK	>100 μ M	>100 μ M	>100 μ M	>100 μ M	-	>100 μ M	
CQIWKQKPDL	516.6	>100 μ M	>100 μ M	1 μ M	-	-	350nM	10nM
CQIWKQKPDLAC	1359	>100 μ M	1 μ M	-	-	-	350nM	10nM
CQIWKQKPDLAC	1448	-	100nM	-	-	-	-	NOTE 1
CQIWKQKPDL	1472.2	-	10nM	-	-	-	-	NOTE 2
SES	357.3	>100 μ M	>100 μ M	-	-	-	-	
KKK	609.8	>100 μ M	-	-	-	-	-	

NOTE 1: IN VIVO EFFECT ABOLISHES MACROPHAGES IN AN IN VIVO RATE INTRADERMAL STUDY INDUCED BY 500 ng MCP-1, 300 g IV, AND 10mg SQ 30 MINUTES PRIOR TO MCP-1, D-ALA (" α ") IS ATTACHED TO D-ASP ("d").

NOTE 2: IN SAME STUDY AS NOTE 1 ABOVE, NO EFFECT ON MACROPHAGES SEEN

FIG. 16C

TITLE: COMPOUNDS AND METHODS TO INHIBIT OR AUGMENT AN INFLAMMATORY RESPONSE
 INVENTOR'S NAME: David J. Grainger, et al.
 SERIAL NO.: 09/150,813 DOCKET NO.: 1543.002US1

23/23



STUDY DESIGN TABLE

GROUP	ANIMAL#	N	RX	RX DOSE/ROUTE T=30 MIN	DERMAL AGONIST	DERMAL AGONIST DOSE (ng IN 50 ul) T=0	HOUR OF SACRIFICE
1	1,2,3	3	PBS	200 ug:LV 200 ug:SQ BACK	PBS LPS MCP-1 MCP-1	0 50 100 500	20-24
2	4,5,6	3	NR58-3.14.3	3 ug:LV 100 ug:SQ BACK	PBS LPS MCP-1 MCP-1	0 50 100 500	20-24
3	7,8,9	3	NR58-3.14.3	30 ug:LV 1 mg:SQ BACK	PBS LPS MCP-1 MCP-1	0 50 100 500	20-24
4	10,11,12	3	NR58-3.14.3	300 ug:LV 10 mg:SQ BACK	PBS LPS MCP-1 MCP-1	0 50 100 500	20-24

FIG. 17